

# UNIVERSITY OF BIRMINGHAM

University of Birmingham  
Research at Birmingham

## Colour and size influences plastic microbead underestimation, regardless of sediment grain size

Nel, Holly A.; Dalu, Tatenda; Wasserman, Ryan J.; Hean, Jeffrey W.

DOI:

[10.1016/j.scitotenv.2018.11.261](https://doi.org/10.1016/j.scitotenv.2018.11.261)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Nel, HA, Dalu, T, Wasserman, RJ & Hean, JW 2019, 'Colour and size influences plastic microbead underestimation, regardless of sediment grain size', *Science of the Total Environment*, vol. 655, pp. 567-570. <https://doi.org/10.1016/j.scitotenv.2018.11.261>

[Link to publication on Research at Birmingham portal](#)

### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

## Accepted Manuscript

Colour and size influences plastic microbead underestimation, regardless of sediment grain size

Holly A. Nel, Tatenda Dalu, Ryan J. Wasserman, Jeffrey W. Hean



PII: S0048-9697(18)34618-7

DOI: <https://doi.org/10.1016/j.scitotenv.2018.11.261>

Reference: STOTEN 29610

To appear in: *Science of the Total Environment*

Received date: 19 August 2018

Revised date: 3 November 2018

Accepted date: 17 November 2018

Please cite this article as: Holly A. Nel, Tatenda Dalu, Ryan J. Wasserman, Jeffrey W. Hean, Colour and size influences plastic microbead underestimation, regardless of sediment grain size. *Stoten* (2018), <https://doi.org/10.1016/j.scitotenv.2018.11.261>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Colour and size influences plastic microbead underestimation, regardless of sediment grain size**

Holly A. Nel <sup>a,b\*</sup>, Tatenda Dalu <sup>c,d</sup>, Ryan J. Wasserman <sup>e,f</sup>, Jeffrey W. Hean <sup>g</sup>

<sup>a</sup> Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

<sup>b</sup> School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, United Kingdom

<sup>c</sup> Department of Ecology and Resource Management, University of Venda, Thohoyandou 0950, South Africa

<sup>d</sup> South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown 6140, South Africa

<sup>e</sup> Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana

<sup>f</sup> School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia

<sup>g</sup> GroundTruth Consulting, 9 Quarry Road, Leonard, Hilton 3245, South Africa.

\*Corresponding author e-mail: hollynel1988@gmail.com

**ABSTRACT**

The quantification of microplastics in environmental samples often requires an observer to determine whether a particle is plastic or non-plastic, prior to further verification procedures. This implies that inconspicuous microplastics with a low natural detection may be underestimated. The present study aimed at assessing this underestimation, looking at how colour (white, green and blue), size (large; ~1000  $\mu\text{m}$  and small; < 400  $\mu\text{m}$ ) and grain size fraction may affect detection. Sediment treatments varying in grain size were inoculated with known quantities of low-density polyethylene microbeads extracted from commercially bought facial scrubs. These microbeads varied in colour and size. Once extracted using a density separation method microbeads were counted. An overall underestimation of 78.59 % may be a result of observer error and/or technical error. More specifically, the results suggested that microbeads varying in colour and size have a different detection probability and that these microbead features are more important in underestimation likelihoods than grain sizes.

**Key words**

microplastics, analytical methods, detection probability, observer error

**Conflict of interest**

There are no conflicts to declare.

## 1. Introduction

Microbeads, an abrasive media, used in personal care products and air-blasting (Gregory, 1996) were identified as a source of microplastic pollution in the 1990s (Zitko and Hanlon, 1991). In the United States alone, trillions of microbeads are discharged into the aquatic environment every day (Rochman et al., 2015). Eriksen et al. (2013) found on average 43 000 particles km<sup>-2</sup> in the Laurentian Great Lakes, while Castañeda et al. (2014) estimated microbead contamination in the St Lawrence River sediment to be ~13 759 particles m<sup>-2</sup>; in both instances, the authors suggested that the primary source of contamination was from commercial products such as facial scrubs.

Unlike microfibers, which are brightly coloured and elongated making them relatively easy to detect, microbeads have a relatively similar shape to natural particles and as a result are thought to be often underestimated (Castañeda et al., 2014; Napper et al., 2015). Furthermore, given that grain size fractionation characteristics vary spatially, and that the potential source microbead size spectrum is considerable (Napper et al., 2015), microbead underestimation levels are highly likely to vary in space. Our understanding of microbead contamination has been supplemented by alternate methods to quantify load within a defined area. One method involved the enumeration of microbeads in popular consumer products; Napper et al. (2015) estimated that between 4 594 and 94 500 microbeads sourced from six brands of facial products alone can potentially pass through the sewerage works. Van Wezel et al. (2016) modelled the potential microbead load entering an aquatic environment using information on product use, sales, population densities, microbead properties and waste management which supported the findings of Van Wezel et al (2016). Similarly, quantification of microplastics in wastewater treatment plant (WWTP) effluent and sludge was used by Rochman et al. (2015) to determine the amount of microbeads entering the surrounding environment.

While the underestimation of microbead densities in sediments is often acknowledged, few studies have assessed the level of this error (but see Conkle et al., 2018). The overarching aim of the present study was to quantify the extent of underestimation using the traditional density separation method. Microbeads varying in colour (white, green, blue) and size (large; ~1000  $\mu\text{m}$  and small; < 400  $\mu\text{m}$ ) were inoculated into sediment of different grain size fractions (<63  $\mu\text{m}$ , 63–125  $\mu\text{m}$ , 125–250  $\mu\text{m}$ , 250–500  $\mu\text{m}$ , 500–1000  $\mu\text{m}$ ). We hypothesised that there would be a difference in the detectability of microbeads varying in colour and size. We also hypothesised that microbead recovery rates would differ significantly among sediment grain size classes.

## 2. Materials and methods

### 2.1. Sediment processing

Sediment was collected from an open quarry (33°18'55.82"S, 26°33'33.39"E) on the floodplain of the Bloukrans River near Grahamstown in 2016. Once in the laboratory, all sediment was dried in ovens at a temperature of 60 °C for 48 hours. Dried sediment was then portioned into 500 g subsamples and passed through a series of stacked sieves, agitated by a mechanical shaker, to fractionate the sediment into various grain sizes. Each subsample was shaken on an EMS-8 Electromagnetic Sieve Shaker (set to a power level of 15) for 60 minutes. The sieves used in the fractionation process of each 500 g subsample were 63, 125, 250, 500 and 1000  $\mu\text{m}$  mesh pore resulting in five size classes of sediment for the experiment, namely: size class 1 (<63  $\mu\text{m}$ ), size class 2 (63–125  $\mu\text{m}$ ), size class 3 (125–250  $\mu\text{m}$ ), size class 4 (250–500  $\mu\text{m}$ ) and size class 5 (500–1000  $\mu\text{m}$ ). The size class 6 (mixed) 500g subsample was comprised of 100g each of size classes 1, 2, 3, 4 and 5, respectively. Once sorted, all sediment was ashed in a furnace at 450 °C for 12 hours to break down any non-geological material. This allowed all

samples to be consistent regarding organic content, thereby removing a potential confound. In addition, the sediment was collected from a field site, which may have been contaminated with microplastics. Exposing the samples to such a high heat made sure no existing plastic was present in the sediment prior to our inoculation. The six sediment size classes were used as sediment treatments.

## *2.2. Experiment set-up and design*

Five replicates (500 g) per sediment treatment were placed in separate 5 L polyethylene containers. Each replicate container for each treatment was then inoculated with the same known quantities of microbeads varying in colour (3; white, green and blue) and size (2; large ( $n = 20$  particles), small ( $n = 200/35/20$  particles)). All microbeads, identified as polyethylene from individual product ingredients list, were removed from commercially available, off-the-shelf, water-based facial cleansers/body scrubs purchased from a grocery store in Grahamstown, South Africa. Once removed microbeads were dried in an oven at a temperature of 60 °C for 48 hours. Large microbeads ranged in size from 900 to 1100  $\mu\text{m}$ . Small white and green microbeads were too small to individually count, therefore, 0.1 g of each was inoculated in individual containers. Three replicates of 0.1 g of small white and green microbead particles were then counted to determine the average number of microbeads in 0.1 g of material and this equated to ~200 white and ~35 green particles. The small white, green and blue microbeads had sizes ranging from 80 to 280; 200 to 400 and 240 to 400  $\mu\text{m}$ , respectively. Measurements of individual microbeads were made using a ZEISS stereo microscope fitted with eyepiece graticule.

The microplastics were then mixed into the sediment by hand for a period of 30 seconds. Salt water at a concentration of 100 g L<sup>-1</sup> (density = 1.07 g cm<sup>-3</sup>) was made using coarse sea salt

and tap water. The saltwater was then filtered through a 20  $\mu\text{m}$  mesh sieve to remove any potential microplastic contaminants that fell within the size-classes of the microplastics used for inoculating treatments. Two litres of filtered saltwater was then added to each treatment, thereafter the solution was vigorously stirred for 5 minutes and the supernatant sieved through a 63  $\mu\text{m}$  mesh following Claessens et al. (2011) and Thompson et al. (2004). This decanting process was repeated five consecutive times to maximise recovery (Coppock et al., 2017). The entire process was also conducted on control treatments for each grain size whereby sediments were not inoculated with any microbeads. Once samples had been decanted microbeads were counted and recorded using a ZEISS stereo microscope at 40 $\times$  magnification by a researcher with no prior knowledge of the inoculated samples. The final number of microbeads found was used to determine the percent particle recovery (%) of each microbead type in each sediment treatment. In the controls, 8 flattened microplastic fragments and no spherical microbeads were found over all 30 treatments. As such, all microbeads enumerated in the study were assumed to be from inoculation rather than sediment contamination from the site of collection.

### *2.3. Statistical analysis*

A factorial Analysis of Variance (ANOVA) was performed using colour, size and grain size as the three independent variables with percent particle recovery (%) as the dependent variable. All statistical analyses were conducted in Using SPSS version 16.0 (SPSS Inc., 2007).

## **3. Results and Discussion**

In this study, large white (mean  $27 \pm 25.5$  %) and green ( $76.5 \pm 18.2$  %) microbeads had a better recovery than their small sized coloured particles, with small white and green microbeads having recovery rates of  $5.5 \pm 2.5$  % and  $8.6 \pm 6.0$  %, respectively (Fig. 1). However, large blue microbeads ( $53.2 \pm 16.9$  %) had a lower recovery than their smaller sized particles ( $109 \pm$



28.2 %). This was, however, due to the overestimation of small blue microbeads. The variables “colour” ( $F = 200.05$ ,  $df = 2$ ,  $p < 0.001$ ) and “size” ( $F = 18.13$ ,  $df = 1$ ,  $p < 0.001$ ) had a significant effect on percent particle recovery. There may also be a concern that grain size impairs visual separation of microplastics, especially as fractionation is a well-documented pre-sorting step (Rocha-Santos et al., 2015) and density separation often results in sediments becoming part of the supernatant. However, “grain size” ( $F = 0.820$ ,  $df = 5$ ,  $p = 0.537$ ) did not have a significant effect on percent particle recovery. Overall, the results suggested that white microplastics and microplastics  $<300 \mu\text{m}$  may be underestimated in studies. This may be due to their inconspicuous colouring and size, which could be further hindered by biofilm growth and discolouration due to aging although this would need to be investigated with additional experiments.

Out of 9 450 inoculated microbeads varying in colour and size, only 2 023 were recovered, with a shortfall of 7 427 microbeads over 30 treatments (Fig. 1). Averaged over the 30 samples, this would equate to a shortfall of 495 microbeads per kg or 78.59 %. Every microbead category, with the exception of small blue microbeads, were underestimated. This study serves as a starting point to begin quantifying and subsequently improving the artefact of observer error.

Observer error is a major concern in microplastic studies as most laboratories still rely on manual sorting and visual identification (Coppock et al., 2017). Even in conjunction with other techniques, such as Fourier-transform infrared spectroscopy (FTIR), visual sorting remains a mandatory step (Hidalgo-Ruz et al., 2012; Coppock et al., 2017). New techniques to minimise observer error are being developed, such as the use of Nile Red (Erni-Cassola et al., 2017; Maes et al., 2017) and Simultaneous Thermal Analysis - Mass Spectrometry (STA-MS;

Dümichen et al., 2015). These studies are still in their infancy and as such, the utilisation of conventional visual identification procedures will likely persist for years to come. The majority of microplastic studies will, therefore, still require an observer to initially decide whether a particle is plastic or non-plastic, prior to further verification procedures.

The low recovery rates could in part be attributed to the density separation method which inadequately removed microbeads from the sediment (technical error). Currently, density separation is the most popular method of extracting microplastics from sediment (Hidalgo-Ruz et al., 2012; Miller et al., 2017), with the web of knowledge article search revealing that in 2018, 16 out of the 19 studies reported using sodium chloride for quantifying microplastics in sediments. Two used sodium iodide (Di and Wang, 2018; Zhang and Liu, 2018), one lithium metatungstate (Eo et al., 2018), one zinc chloride (Lo et al., 2018) and one potassium formate solution (Xiong et al., 2018). Although the solute used varies between studies (see Miller et al., 2017 and Coppock et al., 2017), sodium chloride (NaCl) is still the most commonly used method and hence the one used in this study. While Quinn et al. (2017) estimated a recovery rate of between 85 % and 95 % for sodium chloride, they noted that high density plastics (i.e. PET) were likely missed using this solute. Coppock et al. (2017) on the other hand, stated that NaCl media has a low recovery rate (between 35 and 40 %), which can be compensated for by repeating the decanting method 3 to 5 times, making the method more time consuming. Since the majority of microbeads are made from low-density polyethylene (Cheung and Fok, 2017; Kalčíková et al., 2017), with density values ranging from 0.915 to 0.925 g cm<sup>-3</sup> (Quinn et al., 2017), compared to sodium chloride (1.07–1.2 g cm<sup>-3</sup>), this extraction technique should be effective. However, it cannot be ruled out that these results are in part due to the extraction technique. Additionally, it is apparent that although studies have reported the ineffectiveness of sodium chloride in extracting high-density plastics many studies are still using this method

in hopes that most low-density plastics are recovered; this study suggests that this may not be the case.

#### **4. Conclusions**

Our study suggests that each type and colour of microbead has a different detection probability. The next step would be to consider the issue of microplastic observer error in water samples, which may remove the technical caveat introduced by using the density separation technique and the sorption of microplastics to the polyethylene containers employed in extractions. Future studies would benefit from more replication (>5) as the standard deviation in this study was high. However, it is clear that research into microplastic contamination of the aquatic and terrestrial environments are on the rise. In order for these results to be comparable we should not only focus on standardising sampling and extraction techniques but also enumeration techniques. This will be important when setting up monitoring programmes that are reliant on visual identification. A potential solution may be to employ a global accreditation service. Similar services are used for the identification and enumeration of macroinvertebrates and phytoplankton in both freshwater and marine environments. Although this does not remove observer error, it may reduce it given that it will presumably be conducted by specialists, while also making results more comparable over space and time. Another solution, would be for researchers to quantify observer bias alongside published results allowing readers to better interpret the data.

#### **Acknowledgements**

Financial support for this study was granted by the Rhodes University Grant and Claude Leon Postdoctoral Research Fellowship to TD and partial funding from the National Research Foundation of South Africa to HAN (88414) and RJW (88746). Any opinions, findings,

conclusions or recommendations expressed in this material are those of the authors, and the NRF and Claude Leon Foundation do not accept any liability in this regard.

ACCEPTED MANUSCRIPT

**References**

- Castañeda, R., Avlijas, S., Simard, M., Ricciardi, A., 2014. Microplastic pollution in St. Lawrence River sediments. *Can. J. Fish. Aquat. Sci.* 5, 1–5.
- Cheung, P.K., Fok, L., 2017. Characterisation of plastic microbeads in facial scrubs and their estimated emissions in Mainland China. *Water Res.* 122, 53–61. doi:<https://doi.org/10.1016/j.watres.2017.05.053>
- Claessens, M., Meester, S. De, Landuyt, L. Van, Clerck, K. De, Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Mar. Pollut. Bull.* 62, 2199–2204. doi:[10.1016/j.marpolbul.2011.06.030](https://doi.org/10.1016/j.marpolbul.2011.06.030)
- Conkle, J.L., Báez Del Valle, C.D., Turner, J.W., 2018. Are We Underestimating Microplastic Contamination in Aquatic Environments? *Environ. Manage.* 61, 1–8. doi:[10.1007/s00267-017-0947-8](https://doi.org/10.1007/s00267-017-0947-8)
- Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., 2017. A small-scale, portable method for extracting microplastics from marine sediments. *Environ. Pollut.* 230, 829–837. doi:<https://doi.org/10.1016/j.envpol.2017.07.017>
- Di, M., Wang, J., 2018. Microplastics in surface waters and sediments of the Three Gorges Reservoir, China. *Sci. Total Environ.* 616–617, 1620–1627. doi:<https://doi.org/10.1016/j.scitotenv.2017.10.150>
- Dümichen, E., Barthel, A.-K., Braun, U., Bannick, C.G., Brand, K., Jekel, M., Senz, R., 2015. Analysis of polyethylene microplastics in environmental samples, using a thermal decomposition method. *Water Res.* 85, 451–457. doi:<https://doi.org/10.1016/j.watres.2015.09.002>
- Eo, S., Hong, S.H., Song, Y.K., Lee, J., Lee, J., Shim, W.J., 2018. Abundance, composition, and distribution of microplastics larger than 20  $\mu\text{m}$  in sand beaches of South Korea. *Environ. Pollut.* 238, 894–902. doi:<https://doi.org/10.1016/j.envpol.2018.03.096>

- Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar. Pollut. Bull.* 77, 177–182. doi:10.1016/j.marpolbul.2013.10.007
- Erni-Cassola, G., Gibson, M.I., Thompson, R.C., Christie-Oleza, J.A., 2017. Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20  $\mu\text{m}$ ) in Environmental Samples. *Environ. Sci. Technol.* 51, 13641–13648. doi:10.1021/acs.est.7b04512
- Gregory, M.R., 1996. Plastic scrubbers' in hand cleansers: A further (and minor) source for marine pollution identified. *Mar. Pollut. Bull.* 32, 867–871. doi:10.1016/S0025-326X(96)00047-1
- Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2013. Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environ. Sci. Technol.* 46, 3060–75. doi:10.1021/es2031505
- Kalčíková, G., Alič, B., Skalar, T., Bundschuh, M., Gotvajn, A.Ž., 2017. Wastewater treatment plant effluents as source of cosmetic polyethylene microbeads to freshwater. *Chemosphere* 188, 25–31. doi:https://doi.org/10.1016/j.chemosphere.2017.08.131
- Lo, H.-S., Xu, X., Wong, C.-Y., Cheung, S.-G., 2018. Comparisons of microplastic pollution between mudflats and sandy beaches in Hong Kong. *Environ. Pollut.* 236, 208–217. doi:https://doi.org/10.1016/j.envpol.2018.01.031
- Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Sci. Rep.* 7, 44501.
- Miller, M.E., Kroon, F.J., Motti, C.A., 2017. Recovering microplastics from marine samples: A review of current practices. *Mar. Pollut. Bull.* 123, 6–18. doi:https://doi.org/10.1016/j.marpolbul.2017.08.058

- Napper, I.E., Bakir, A., Rowland, S.J., Thompson, R.C., 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar. Pollut. Bull.* 99, 178–185. doi:10.1016/j.marpolbul.2015.07.029
- Quinn, B., Murphy, F., Ewins, C., 2017. Validation of density separation for the rapid recovery of microplastics from sediment. *Analytical Methods*. 9, 1491–1498. doi:10.1039/c6ay02542k
- Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. *TrAC Trends Anal. Chem.* 65, 47–53. doi:https://doi.org/10.1016/j.trac.2014.10.011
- Rochman, C.M., Kross, S.M., Armstrong, J.B., Bogan, M.T., Darling, E.S., Green, S.J., Smyth, A.R., Veríssimo, D., 2015. Scientific Evidence Supports a Ban on Microbeads. *Environ. Sci. Technol.* 49, 10759–10761. doi:10.1021/acs.est.5b03909
- SPSS Inc., 2007. SPSS Release 16.0.0 for Windows. Polar Engineering and Consulting. SPSS Inc., Chicago (IL).
- Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? *Science* (80-. ). 304, 838 LP-838.
- van Wezel, A., Caris, I., Kools, S.A.E., 2016. Release of primary microplastics from consumer products to wastewater in the Netherlands. *Environ. Toxicol. Chem.* 35, 1627–1631. doi:10.1002/etc.3316
- Xiong, X., Zhang, K., Chen, X., Shi, H., Luo, Z., Wu, C., 2018. Sources and distribution of microplastics in China's largest inland lake – Qinghai Lake. *Environ. Pollut.* 235, 899–906. doi:https://doi.org/10.1016/j.envpol.2017.12.081

Zhang, G.S., Liu, Y.F., 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. *Sci. Total Environ.* 642, 12–20.  
doi:<https://doi.org/10.1016/j.scitotenv.2018.06.004>

Zitko, V. & Hanlon, M. (1991). Another source of pollution by plastics: skin cleaners with plastic scrubbers. *Mar. Pollut. Bull.* 22, 41-42.

### **Addendum**

#### **Figure captions**

**Fig. 1.** Microbead recovery levels (%) in different sediment grain sizes: (a) white, (b) green and (c) blue microbeads.



**Highlights:**

- Microbeads, found in cosmetics, vary in colour and size.
- Detection probabilities were significantly different between microbeads.
- Microbead characteristics may explain underestimation in *in situ* sampling.

ACCEPTED MANUSCRIPT

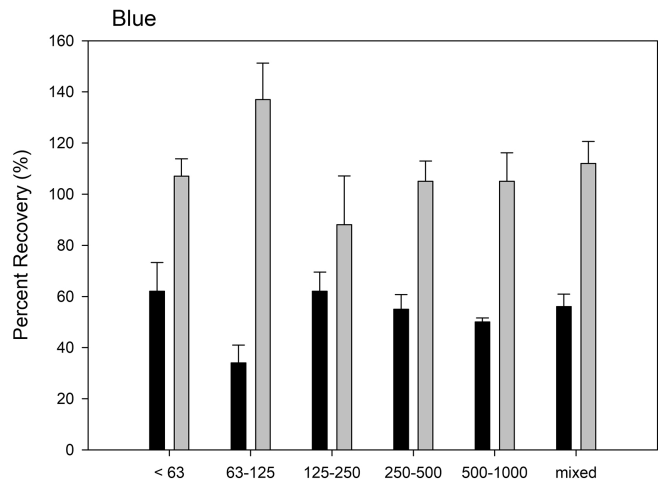
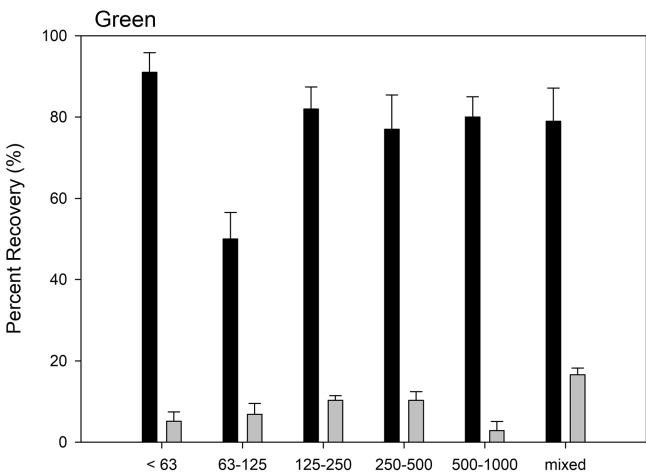
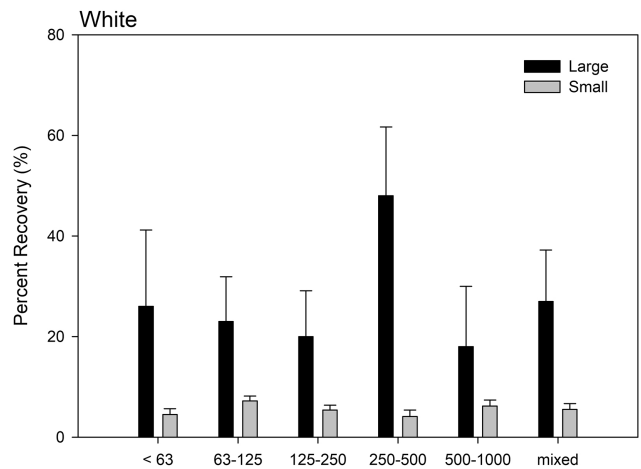


Figure 1